THE EFFECT OF ALKALIES ON THE OXYGEN CONSUMPTION AND SUSCEPTIBILITY OF *PLANARIA DOROTOCEPHALA*.[1]

LOUISE A. ANDERSON,

Hull Zoölogical Laboratory, University of Chicago.

I.

Introduction.

Considerable work has been done in this laboratory by Child and his students on the effect of chemical and physical agents on the modification of development and regeneration of organisms. For the interpretation of these results it is desirable that the action of the more commonly used chemicals on the rate of some metabolic process, such as respiration, be determined directly. Some work has already been accomplished along these lines, the effects of several substances on the rate of oxygen consumption having been tested: cyanides (Hyman, '19*a*), anesthetics (Buchanan, '22), caffein (Hinrichs, '24), and acids (Hyman, '25). The present paper is a contribution to this line of work and consists mainly of a study of the action of alkalies on the rate of oxygen consumption of *Planaria dorotocephala*. Hyman ('25) has already shown that acidification of natural water decreases the rate of oxygen consumption of *Planaria*. The question of the effect of increased alkalinity of natural water seemed of interest.

A number of investigations are available on the effect of increased alkalinity on various biological processes, such as activity, growth and development, and respiratory rate. Loeb ('98) found that the rate of embryonic development of *Arbacia* and *Fundulus* is accelerated in slightly alkaline solutions. Moore, Roaf, and Whitley ('05) also noted that bases in low concentrations favor growth and cell division in the fertilized eggs of *Echinus esculentus*; but Whitley ('05) failed to obtain any accelerating effect of

[1] Accepted as a thesis for the degree of Master of Science in the department of zoölogy of the University of Chicago.

alkaline solutions on the development of the teleost *Pleuronectes*. The fertilizability of *Arbacia* and *Asterias* eggs is increased by short exposures to alkaline sea-water according to Smith and Clowes ('24). Accelerations of activity by alkaline solutions have been observed by Dale ('13) in *Paramecium* and by Gray ('24) in the frontal cilia of *Mytilus edulis*.

Direct measurements of respiratory rate in alkaline solutions have yielded diverse results. In certain bacteria Brooks ('21, '22) noted the maximum rate of carbon dioxide production at or near neutrality with progressive decline in the rate with increasing alkalinity. The respiratory rate of the mold *Penicillium chrysogenum* is decreased 60 per cent. upon increasing the alkalinity of the medium from neutrality to pH 8.8 with NaOH (Gustafson, '20). According to Loeb and Wasteneys ('11) the rate of oxygen consumption of *Arbacia* eggs is increased 20–30 per cent. by raising the alkalinity of the sea-water with NaOH to pH 10.0 and increased 100 per cent. by raising it from pH 10.0 to 10.9. Similar results were obtained when ammonia was used. Thunberg ('09) found a decrease in the carbon dioxide production of excised frog muscle in solutions of NaOH, $Ca(OH)_2$, and $Mg(OH)_2$. Waldbott ('24) noted a slight acceleration of the respiratory metabolism of humans after the ingestion of alkaline solutions. The carbon dioxide production of tadpoles is increased in solutions of potassium and sodium hydroxide, but the rate of regeneration of the tail is retarded (Jewell, '20); Jewell regarded the increased respiratory metabolism as of a destructive nature.

From this review of the literature it is evident that both retardation and acceleration of biological processes result from exposure of organisms to alkaline solutions. In general, in the case of animal materials, an acceleration of growth and development and of respiratory metabolism in alkaline solutions has been found in the majority of cases.

Oxygen Consumption Experiments.

1. *Methods*.—The experiments consisted in determining the rate of oxygen consumption of planarians first in normal water

and then in water made alkaline to the desired degree by the addition of sodium or ammonium hydroxide. The general method of procedure was that described by Hyman ('19*a*). The species used was *Planaria dorotocephala*. Each experimental lot consisted of about 150 worms, 18–20 mm. long; these were selected from the laboratory stock and placed in a 500 cc. Erlenmeyer flask, in which they remained for a considerable time, being used in a number of experiments. They were fed in these flasks at intervals. At least three or four days were allowed to elapse after each feeding before the worms were used for experiment in order to avoid the increased respiratory rate consequent upon feeding (Hyman, '19*b*). The worms were fed sufficiently often to avoid any effects of starvation. From time to time new lots of worms were selected.

The water used was well water, an analysis of which is given by Hyman ('25). The alkaline water was prepared by adding enough sodium or ammonium hydroxide from stock solutions to raise this water to the desired alkalinity. The pH was determined with phenol red and thymol blue indicators by comparison with standard sets. At alkalinities greater than pH 8.6 a precipitate of calcium carbonate formed in the water. While there was no indication that this precipitate in any way affected the results, it was thought best to avoid it. This was done either by allowing the precipitate to settle and then decanting or by using carbonate-free water. This was prepared by adding 2 cc. concentrated HCl to eight liters of well-water and bubbling air through the water for 24 hours or more. That the rate of oxygen consumption of *Planaria dorotocephala* is the same in carbonate-free as in untreated well-water had previously been determined by Hyman ('25) and was verified in the present experiments.

Throughout each respiratory test, the flasks containing the worms and the blanks were kept in a large water bath at a temperature of 20° C. ± 0.5°. This was covered during the test. This darkening of the flasks together with the fact that the worms were kept continuously in the flasks was sufficient to eliminate movement. The worms remained very quiet throughout the oxygen consumption tests and the results cannot be ascribed to any differences in motor activity.

Two types of experiments were carried out: short exposures of a few hours to alkaline solutions, and long exposures of a week or more. Three experiments, that is, three flasks of worms, were generally carried on simultaneously.

2. *Short Time Experiments.*—In these experiments the rate of oxygen consumption of the worms was tested for two successive hours in normal well-water, pH 7.6 to 7.8. The water was then made alkaline by adding NaOH in varying amounts, giving a pH of 8.0 to 9.2 by intervals of 0.2 pH, or by adding NH_4OH similarly to alkalinities varying from pH 8.0 to 8.8. The rate of oxygen consumption was then tested in this alkaline water during the first third, and generally also fifth hours of exposure to it, freshly made alkaline water being used for each determination. As stated above, carbonate-free water was employed for alkalinities greater than pH 8.6. Twenty-three experiments were performed with sodium hydroxide and twelve with ammonium hydroxide. Table I. gives a typical experiment with each pH used for each of the two alkalies. The average per cent. of increase is based on all of the figures obtained in the alkaline solution. Table II. presents a summary of all of the short time experiments showing also the minimum, maximum, and average change in respiratory rate These tables show that the respiratory rate was sometimes decreased in alkaline solution but was more generally accelerated The normal variation in respiratory rate, based on twenty experiments where the respiration was determined in normal water for two successive hours, was 7 per cent. Only figures showing more than 7 per cent. alteration of the respiratory rate are therefore significant. From Table II. it can be seen that the higher alkalinities give generally a significant acceleration of the rate of oxygen consumption.

The worms would not survive alkalinities greater than those given in the table. They would live indefinitely in water made alkaline by NaOH to pH 9.0, but only twenty-four hours at pH 9.2. They were immediately killed in NH_4OH at pH 9.0. These results indicate that the action of alkali cannot be attributed solely to the hydroxyl ion.

It should be stated that the oxygen consumption of *Planaria* is independent of the oxygen content of the water at all ordinary oxygen concentrations (between 7.0 and 2.0 cc. per liter, at least). The oxygen concentrations employed in the experiments was such (5 to 7 cc. per liter) that there is no possibility that the reduction in oxygen content by the worms during the experiment could have the slightest effect upon the amount of oxygen consumed.

Table I.

Results of a Typical Experiment, Short Time, at Each pH Used.

Figures represent cc. of oxygen consumed per hour.

	NaOH						NH_4OH			
Normal respiration in water. pH 7.6–7.8.										
	0.21	0.24	0.21	0.22	0.26	0.26	0.29	0.26	0.26	0.31
Respiration in alkaline water.										
pH	8.0	8.4	8.6	8.8	9.0	9.2	8.0	8.4	8.6	8.8
1st hr.	0.23	0.26	0.33	0.26	0.27	0.33	0.29	0.28	0.29	0.39
3d hr.	0.25	0.30	0.24	0.28	0.33	0.34	0.30	0.26	0.26	0.37
5th hr.	—	—	—	—	0.30	0.30	—	0.30	0.28	0.39
Average per cent. of increase of each of above.										
	14	16	32	22	15	20	1.5	7.6	6	23

Table II.

Average Percentages of Increase and Decrease Based on Total of All Short Experiments Done at Each pH.

Figures are ± per cent. from the normal.

	NaOH						NH_4OH			
pH	8.0	8.4	8.6	8.8	9.0	9.2	8.0	8.4	8.6	8.8
Total no. exp.	3	5	3	6	3	3	3	3	3	3
Increase and decrease in respiration rate from normal.										
Min.	−12	−12	+11	− 4	+ 3	− 7	−20	−18	−10	+19
Max.	+19	+28	+61	+86	+26	+58	+ 3	+15	+17	+32
Av. of all exp.	+ 6	+14	+32	+22	+17	+33	− 7	+ 3	+ 5	+25

The results of all of the short time experiments are given in graphic form in Fig. 1, made from the data given in Table II. The rate of oxygen consumption is given on the ordinate, normal respiration in untreated water being taken as 100, respiration in alkaline water as per cent. decrease or increase from this. The alkalinity in terms of pH is given on the abscissa. The solid line represents the results with NaOH, the dashed line with NH_4OH. As already explained only differences greater or less than 7 per cent can be taken as significant. In the case of NaOH the experiments at pH 8.4 to 9.2 show on the average an increase above the normal variation, but there is apparently no relation between amount of acceleration and degree of alkalinity. The drop at pH 8.8 and 9.0 is probably not significant. In the case of ammonia the curve rises steadily with increasing alkalinity but probably only the increase at 8.8 is significant.

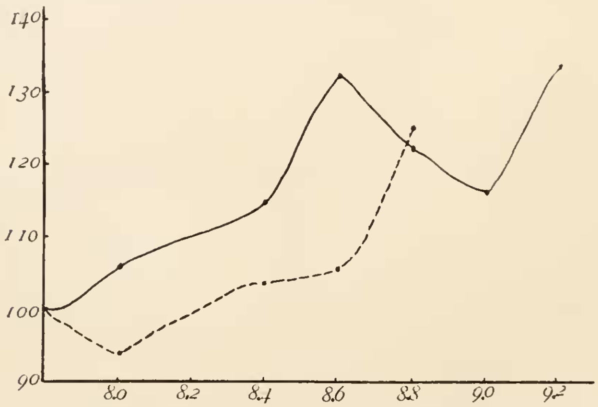

FIG. 1. Graph compiled from the average percentages of increase and decrease in respiration rate of all the short time experiments. See Table II. Per cent. of increase or decrease, normal respiration being taken as 100, on the ordinate and pH on the abscissa. Heavy line shows experiments in NaOH and broken line the experiments in NH_4OH.

3. *Long Time Experiments.*—These experiments consisted in determinations of the rate of oxygen consumption at frequent intervals in worms exposed continuously to alkaline water for periods of one to two weeks. Considerable difficulty was encountered in these experiments because the water would not remain at a definite alkalinity on account of the carbon dioxide given off by the animals. Various methods of keeping the water alkaline were suggested and were tried out. The carbonate-free water could not be used in the long time experiments, as, being unbuffered, it quickly became acid from the carbon dioxide given off by the

animals. Buffering this water with borax proved effective as a means of maintaining it at a definite alkalinity but the borax killed the worms in several days. Bubbling air through the flasks in the hope of removing the carbon dioxide emanating from the animals was not effective. When all of these methods failed, it became necessary to use the ordinary well water and to change it at frequent intervals. This proved fairly satisfactory. Boiling this water to remove dissolved carbon dioxide was of some help. The oxygen consumption of the worms was determined first in untreated well-water, pH 7.6 to 7.8. The water was then made alkaline to pH 9.0 with NaOH, decanted from the precipitate of $CaCO_3$ when necessary, and the worms kept in such water for one to two weeks continuously. The water was changed two or three times in 24 hours. Between changes, it became somewhat less alkaline but never fell below pH 8.6 throughout these long time experiments. The rate of oxygen consumption was determined every other day or every third day, always of course at pH 9.0. Owing to the fact that the worms lost weight during the experiment, because of starvation, for they were not fed throughout the period of experiment, it was necessary to weigh them in order to compare the rate of oxygen consumption at different intervals. The figures therefore represent the cc. of oxygen consumed per gram per hour. At the end of the experiment, the respiratory rate in normal water was again tested.

The results of three long time experiments which lasted one week are given in Table III., and of three more which lasted two weeks, in Table IV. In Table III., the oxygen consumption was determined first in normal water, then immediately in alkaline water (ph 9.0), then every other day in the alkaline water, and finally on the seventh day after the last test in alkaline water, again immediately in normal water. As shown in the table there was a marked acceleration of the respiratory rate during the latter part of the stay in alkaline water; this acceleration was immediately lost on return to untreated water, on the seventh day. The data in Table IV. give a similar result: acceleration during the exposure to alkaline water, immediate drop on return to untreated water.

Table III.

Table Showing Results of Long Time Experiments in Boiled Well Water, pH Raised to 9.0 with NaOH.

The pH never went below 8.6. Results expressed in cc. oxygen consumed per gram of animals per hour.

	1.	2.	3.	Av. % Increase 3 Exp.
I. day. Water pH 8.0	0.32	0.30	0.29	Normal. 100%
I. day. NaOH pH 9.0	0.28	0.28	0.30	− 5.5
III. day. NaOH pH 9.0	0.28	0.29	0.29	− 5.2
V. day. NaOH pH 9.0	0.44	0.26	0.41	+ 22.0
VII. day. NaOH pH 9.0	0.39	0.37	0.45	+ 33.0
VII. day. Water pH 8.0	0.24	0.26	0.25	− 17.0

Table IV.

Table Showing Results of Long Time Experiment in Ordinary Well Water Decanted off Precipitate.

The pH was raised to 9.0 with NaOH. The pH never went below 8.6. Results expressed in cc. oxygen consumed per gram of animals per hour.

	1.	2.	3.	Av. % Increase 3 Exp.
I. day. Water pH 8.0	0.21	0.18	0.19	Normal. 100%
I. day. NaOH pH 9.0	0.23	0.20	0.21	+ 10
III. day. NaOH pH 9.0	0.29	0.26	0.28	+ 43
VII. day. NaOH pH 9.0	0.24	0.23	0.24	+ 22
X. day. NaOH pH 9.0	0.24	0.23	0.21	+ 17
XIV. day. NaOH pH 9.0	0.27	0.29	0.23	+ 36
XIV. day. Water pH 8.0	0.23	0.20	0.18	+ 5

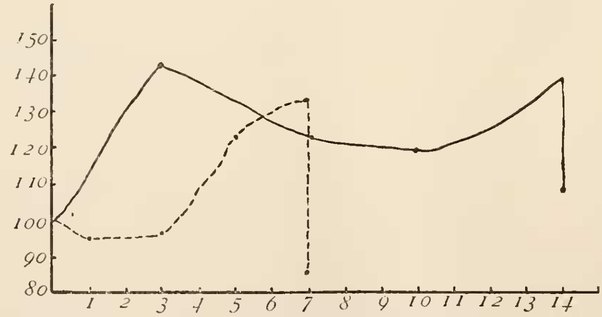

Fig. 2. Graph compiled from the data presented in Tables III. and IV. showing the results of long exposures of worms to NaOH solution, pH 9.0–8.6. Per cent. of increase or decrease, normal respiration being taken as 100, on the ordinate and number of days on the abscissa. Heavy line represents experiment in ordinary water and broken line experiment in boiled well water. The drop on the last day in each experiment shows the drop in respiration from NaOH solution to watei.

The data given in Tables III. and IV. are graphed in Fig. 2, the solid line representing the data of Table IV., the dashed line, of Table III. The graph shows an acceleration of the rate of oxygen consumption during the greater part of the exposure to alkali, with an immediate drop at the end of each experiment on return to normal water.

Susceptibility Experiments.

The susceptibility method of determining differences in oxidative rate in different parts of the same organism and between different individuals of the same species was devised by Child and has been used extensively in this laboratory by him and his students. The method is explained by Child ('24). Briefly, the organisms to be compared are placed in toxic solutions of proper concentration or are exposed to lethal conditions and the various stages in disintegration are recorded. In general, the higher the oxidative rate, the shorter is the time before disintegration begins and the more rapidly does the disintegration progress. The method is therefore a rough means of comparing the relative rates of respiratory metabolism of comparable animals. Since the experiments on oxygen consumption had shown that the respiratory rate of *Planaria* is increased in alkaline solutions it seemed of interest to determine whether or not worms so accelerated would be more susceptible to chemical and physical agents than those respiring normally in ordinary water. A few experiments were tried to test out this point. Worms which had been kept for several hours in water made alkaline to pH 9.0 with NaOH were compared as to their susceptibility to toxic solutions and conditions while still in alkaline solution with control worms kept and tested in ordinary water or in solutions made up in ordinary water (pH about 8.0).

Comparison of susceptibility differences between different lots of worms is best accomplished by recording the progress of disintegration in certain arbitrarily selected stages. In the following experiments seven stages of disintegration were chosen: (1) worm entirely intact; (2) disintegration at the margins of the head; (3) from the end of marginal disintegration to complete disintegration of the head; (4) from the end of stage 3 to completion of disintegration half way back to the mouth; (5) from the end of stage 4 to completion of disintegration to the level of the mouth; (6) from the end of stage 5 along the margins to the posterior end of the first zooid; (7) from the end of stage 6 to complete disintegration of the first zooid. The death of the secondary zooids is not considered. Ten animals are usually employed in each test, and the number of animals in each stage of disintegration is recorded hourly. The results are graphed by giving to each stage a numerical value, beginning with zero for stage 7, complete disintegration, and adding ten for each stage, intact animals being assigned the value of 60. The numerical value assigned to each stage is then multiplied by the number of animals in each stage at each observation. These values for the ten animals are then added together and divided by ten; the quotient thus gives the average stage of disintegration of the ten animals at the particular time of observation. Thus if at one observation, three animals were in stage 2, four in stage 3, and three in stage 4, their values would be 100,160, and 120, respectively, making a sum of 380; this divided by ten gives 38, or the average stage of disintegration reached, at the time in question.

1. *Experiments with Chemicals.*—To test the effect of alkalies on susceptibility to toxic chemical solutions, it was necessary of course to choose chemicals that were in themselves neutral. Anæsthetics were selected as the most convenient. Ethyl alcohol, 4 per cent., and chloretone, 0.1 per cent., were used. The anæsthetics for the control set of worms were made up in ordinary well water, those for the experimental set in the same water, made alkaline to pH 9.0 with NaOH. The results are shown in Figs. 3 and 4, being graphed according to the method explained above. Fig. 3 represents the results with alcohol, Fig. 4 with chloretone. In each graph, the solid line is the rate of disintegration of the control worms in ordinary water, the dashed line, the experimental worms in alkaline water. In each case, the disintegrating action of the anæsthetic is seen to be more rapid in alkaline solution.

2. *Experiments with Lack of Oxygen.*—When chemical agents are used the question of their penetrability is involved and complicates the interpretation of the results. For this reason it was thought advisable to kill the animals by other than chemical means

and determine the effect of alkalinity on the time of death. Lack of oxygen was one condition that seemed suitable for a test. A petri dish was divided into three compartments by two paraffin walls. A powder made by grinding up pyrogallic acid and sodium

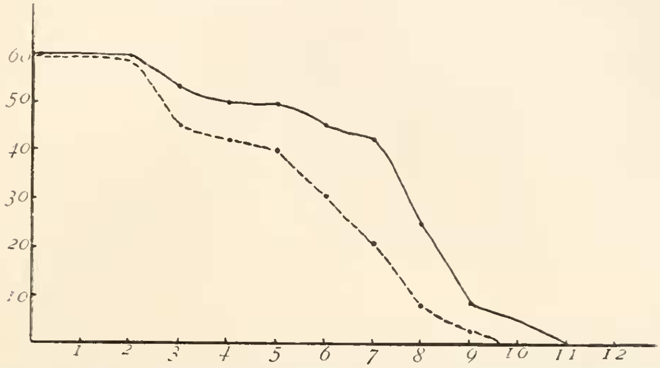

FIG. 3. Graph of disintegration gradient of worms exposed to NaOH solution and tested in alkaline alcohol (broken line) and worms not exposed to alkali and tested in neutral alcohol (heavy line). Alcohol used was 4 per cent. Hours on the abscissa and stages in disintegration on the ordinate. (See text for stages.)

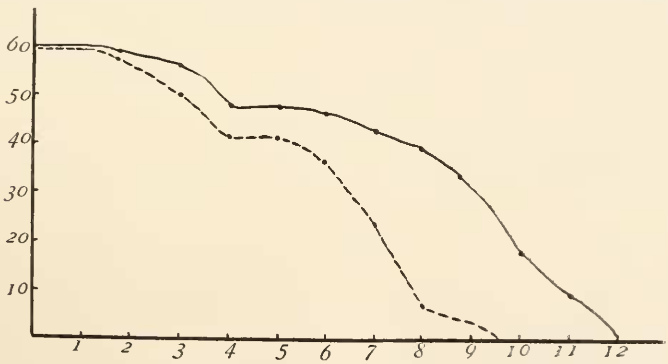

FIG. 4. Graph of disintegration gradient of worms exposed to NaOH solution and tested in alkaline chloretone (broken line) and worms not exposed to alkali and tested in neutral chloretone (heavy line). Chloretone used was 0.1 per cent. Hours on the abscissa and stages in disintegration on the ordinate. (See text for stages.)

hydroxide was placed in the center between the two paraffin walls. On one side of this were placed ten to twenty worms in ordinary water (pH 8.0); and on the opposite side in the third compartment, an equal number of worms of the same size and physiological condition in alkaline water (pH 9.0). A cover was sealed on airtight. As the oxygen was absorbed by the alkaline pyrogallate, the worms began to die. The results of one such experiment are graphed in Fig. 5, the solid line representing the control worms,

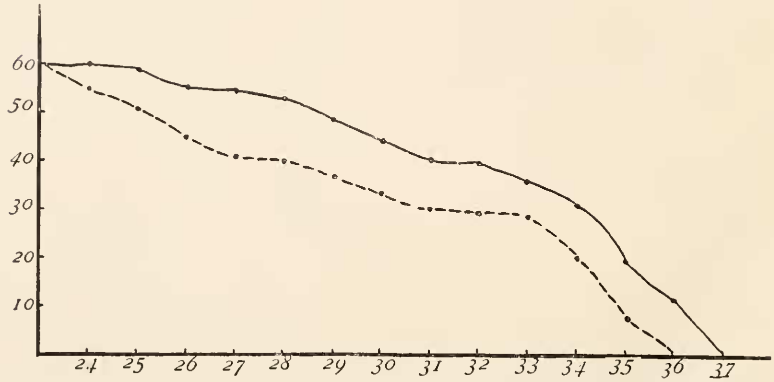

FIG. 5. Graph of disintegration gradient of worms exposed to NaOH solution and tested in alkaline solution in lack of oxygen experiment. Heavy line represents worms in ordinary water and broken line worms exposed to alkali. Record was not taken until disintegration began, *i.e.*, the twenty fourth hour. Hours are on the abscissa and stages of disintegration on the ordinate.

the dashed line, the experimental worms. In several experiments of this kind it was always found that the worms died faster from lack of oxygen at pH 9.0 than at pH 8.0.

3. *Experiments with Ultraviolet Radiation.*—Comparable lots of worms were exposed to ultraviolet radiation from a Cooper-Hewitt machine for a duration known to be lethal, one lot at pH 8.0, the other at pH 9.0. The worms were continually agitated during the exposure to insure uniform radiation. In all trials, the worms in the more alkaline water died more rapidly than those at the lower alkalinity. Death of course occurs only some hours after exposure to the radiation.

These experiments indicate that planarians are more susceptible to toxic chemicals and to lethal conditions when exposed to them in water of increased alkalinity, which is not in itself injurious.

4. *Susceptibility to Alkali.*—The question was raised whether the worms are able to acclimate to increased alkalinity. The long time experiments did not suggest that this was the case within two weeks' exposure, since the acceleration of the respiratory rate

endured through the period of the experiments. A further test by the susceptibility method was suggested. If any acclimation occurred, worms that had been kept in a non-injurious concentration of alkali should be less susceptible to a lethal concentration of alkali than control worms. Stocks of worms were kept in water made alkaline to pH 8.8 and 9.0, respectively, with NaOH, and were compared as to susceptibility to a higher concentration of NaOH with control worms living in ordinary water, pH 8.0. Tests were made every third day over a period of ten days. No difference in susceptibility between experimental and control worms was found. The resistance of *Planaria* to alkali was, therefore, not increased by continuous exposure, up to ten days, to alkaline water.

Discussion.

The experiments reported in this paper show that in general the rate of oxygen consumption is accelerated in water made more alkaline than normal, within physiological limits, by sodium or ammonium hydroxide. This increase in the respiratory rate lasts as long as the worms remain in the alkaline water, at least up to two weeks. Upon return to water of normal alkalinity, the respiratory rate drops at once. No evidence of any acclimation to the alkaline environment appeared in the course of the experiments. The oxygen consumption remained at a supernormal figure during continued exposure to the increased alkalinity. Susceptibility tests also showed no increased tolerance to alkali as a result of living for some time in water of increased alkalinity.

The question of the cause of the accelerated respiratory rate in alkaline water is of interest but the present experiments throw no light on the matter. It is rather generally accepted that alkalies increase permeability or have some other surface action (*e.g.*, Osterhaut, '14, and Warburg, '10). Such surface changes might well be the cause of the acceleration of the respiratory rate. It is not probable that penetration of the alkali into the interior is a factor in the acceleration for although ammonia penetrates cells readily it is believed that sodium hydroxide does not penetrate until the surface is actually injured. Since both alkalies caused an increase in the rate of oxygen consumption, the effect appears to be a surface one.

Not only is the oxygen consumption of *Planaria* increased with exposure to alkaline solutions but also the susceptibility to toxic agents and conditions is greater when such agents and conditions are applied in alkaline water. In the case of chemical agents, this increased susceptibility in alkaline solution might be ascribed to increased permeability. This explanation does not seem applicable, however, to the result with lack of oxygen and ultraviolet radiation. Exposure to these conditions is more rapidly lethal in water of increased alkalinity than in normal water. It seems necessary to conclude that the increased susceptibility of worms to chemicals and to toxic conditions when exposed in water of increased alkalinity is in some way associated with the accelerated metabolism of the animals in alkaline water. Susceptibility is thus again indicated as a method of measuring roughly differences in general metabolic rate.

Summary.

1. The general result of exposing *Planaria dorotocephala* to water whose alkalinity is increased from pH 7.6 or 7.8 to 8.0 to 9.2 by addition of NaOH or to 8.0 to 8.8 by addition of NH_4OH is an increase in the rate of oxygen consumption, whether the exposure is for long or short periods.

2. The increase lasts as long as the planarians remain in the alkaline water (longest experiment, two weeks). A return to the normal or to a lower rate (probably result of starvation) occurs at once when the animals are returned to water of the original pH.

3. The resistance of planarians to lethal concentrations of alkali is not altered by long exposure (ten days) to non-injurious concentrations of alkali.

4. The susceptibility of planarians to toxic chemical solutions, to lack of oxygen, and to ultraviolet radiation is greater when they are exposed to these conditions at pH 9.0 than when exposed at ordinary alkalinity of normal water (pH 7.8 to 8.0).

I desire to express my thanks to Professor C. M. Child and Dr. L. H. Hyman under whose direction the work was done.

BIBLIOGRAPHY.

Brooks, M. M.

'21 The Effect of Hydrogen Ion Concentration on the Production of Carbon Dioxide by *Bacillus butyricus* and *Bacillus subtilis*. Jour. of Gen. Physiol., Vol. 4, pp. 177–186.

'22 The Effect of Hydrogen Ion Concentration on the Production of Carbon Dioxide by the Tubercle Bacillus. Amer. Rev. of Tuber., Vol. 6, pp. 369–376.

Buchanan, J. W.

'22 The Control of Head Formation in *Planaria* by Means of Anesthetics. Jour. Exp. Zoöl., Vol. 36, No. 1.

Child, C. M.

'24 Physiological Foundations of Behavior.

Dale, D.

'13 On the Action of Electrolytes on *Paramecium*. Jour. of Physiol., Vol. 46. pp. 129–140.

Gray, J.

'24 The Mechanism of Ciliary Movement. The Relation of Ciliary Action to Oxygen Consumption. Proc. Roy. Soc. London, Vol. 96 B, pp. 95–115.

Gustafson, F. G.

'20 Comparative Studies on Respiration. XI. The Effect of Hydrogen Ion Concentration on the Respiration of *Penicillium chrysogenum*. Jour. of Gen. Physiol., Vol. 2, pp. 617–626.

Hinrichs, M. A.

'24 A Study of the Physiological Effects of Caffein upon *Planaria dorotocephala*. Jour. Exp. Zoöl., Vol. 40, No. 2.

Hyman, L. H.

'19*a* On the Action of Certain Substances on Oxygen Consumption. II. Action of Potassium Cyanide on *Planaria*. Amer. Jour. of Physiol., Vol. 48, No. 3.

'19*b* Physiological Studies on *Planaria*. I. Oxygen Consumption in Relation to Feeding and Starvation. Amer. Jour. of Physiol., Vol. 49, No. 3.

'25 On the Action of Certain Substances on Oxygen Consumption. VI. The Action of Acids. BIOL. BULL., Vol. 49, No. 4.

Jewell, M.

'20 The Effects of Hydrogen Ion Concentration and Oxygen Content of Water Upon Regeneration and Metabolism of Tadpoles. Jour. Exp. Zoöl., Vol. 30, pp. 461–507.

Loeb, J.

'98 Über den Einfluss von Alkalien und Säuren auf die embryonale Entwickelung und das Wachstum. Arch. f. Entw'mech., Vol. 7, pp. 631–641.

Loeb, J., and Wasteneys, H.

'11 Die Beeinflussung der Entwicklung und der Oxydationsvorgänge im Seeigelei (*Arbacia*) durch Basen. Biochem. Zeitsch., Vol. 37, pp. 410–423.

Moore, B., Roaf, H. E., and Whitley, E.

'05 On the Effects of Alkalies and Acids and of Alkaline and Acid Salts, upon Growth and Cell Division in the Fertilized Eggs of *Echinus esculentus*. Proc. Roy. Soc. London, Vol. 77B, pp. 102–136.

Osterhout, W. J. V.

'14 The Effect of Alkali on Permeability. Jour. of Biol. Chem., Vol. 19, pp. 335–343.

Smith, H. W., and Clowes, G. H. A.

'24 The Influence of Hydrogen Ion Concentration on Unfertilized *Arbacia*, *Asterias*, and *Chætopterus* Eggs. Biol. Bull., Vol. 47, pp. 304–321.

Thunberg, T.

'09 Studien über die Beeinflussung des Gasaustausches des überlebenden Froschmuskels durch verschiedene Stoffe. Skand. Archiv. f. Physiol., Vol. 23, pp. 154–161.

Waldbott, G.

'24 Über den Einfluss von Säuren, Alkalien, und Neutralsalzen auf den respiratorischen Stoffwechsel des Menschen. Deut. Archiv. f. Klin Med., Vol. 143, pp. 325–333.

Whitley, E.

'05 A Note on the Effect of Acid, Alkali, and certain Indicators in Arresting or otherwise Influencing the Development of *Pleuronectes platessa* and *Echinus esculentus*. Proc. Roy. Soc. London, Vol. 77 B, pp. 137–149.